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(54) Title: SUPPRESSION OF NUCLEAR FACTOR-$_k$B DEPENDENT PROCESSES USING OLIGONUCLEOTIDES

(57) Abstract

Antisense oligonucleotides which hybridize with nuclear factor-$_k$B(NF-$_k$B) mRNA and methods of using these oligonucleotides are provided.

Among those disorders which are linked to activation of NF-κB is leukemia caused by the retrovirus HTLV-1. Human T-cell leukemia virus (HTLV-1) is recognized as the etiologic agent of the human malignancy, adult T cell leukemia (B.J. Poiesz, *Proc. Natl. Acad. Sci. USA*, 77:7415, 1980; D.J. Slamon, *et al., Science*, 226:61, 1984; W.C. Goh, *et al., ibid.* 227:1227, 1985).

Circumstantial data has implicated the HTLV-I encoded tax gene in leukemogenesis. This gene encodes a 40kD protein that causes transcriptional transactivation of viral gene expression and also activates expression of certain cellular genes that are important for growth (A.J. Cann, *et al., Nature* 318:571, 1985; B.K. Felber, *et al., Science*, 229:675, 1985; J. Fujisawa, *et al., Embo Journal* 5:713, 1986). *In vitro* studies have demonstrated that tax can activate the promoters of the interleukin 2 receptor (IL-2R) a-chain, GM-CSF, fos, PDGF, IL-6, NGF, TGF-β, HIV LTR as well as its own LTR (D.J. Slamon *et al., Science*, 226:61, 1984; W.C. Goh *et al., ibid.*, 227:1227, 1985; J. Inoue, *et al., Embo Journal* 5:2883, 1986; S. Miyatake, *et al., Mol. Cell. Biol.* 8:5581, 1988; K. Nagata, *et al., J. Virol.* 63:3220, 1989; L. Ratner, *Nucleic Acid Research*, 17:4101, 1989; J. Sodroski, *et al., Science*, 228:1430, 1985; J.E. Green, *et al., Mol. Cell. Biol.* 11:4635, 1991; S.J. Kim, *et al., J. Exp. Med.*, 172:121, 1990; E. Bohnlein, *et al., J. Virol.*, 63, 1578, 1988). *In vitro* studies of tax effects on gene expression, have demonstrated two independent pathways for its action on transcription (M.R. Smith and W.C. Green, *J. Clin. Invest.* 87:761, 1991). The first affects the family of nuclear transcription factors related to c-rel which bind to NF-κB sites and are important for the normal activation of lymphocytes. NF-κB response sequences occur in a number of genes including the HIV LTR (G. Nable, *et al., Nature* London, 326:711, 1987) and the IL-6 promoter (T.A. Libermann, *et al., Mol. Cell. Biol.*, 10:2327, 1990). The heterodimer composed of the p50 and p65 rel related proteins have been shown to affect the transcription of many of these genes (P.A. Baeuerle, *Biochem. Biophys. Acta*, 1072:63, 1991). The other effect of tax is thought to be NF-κB independent,

SUPPRESSION OF NUCLEAR FACTOR-$_\kappa$B DEPENDENT PROCESSES USING OLIGONUCLEOTIDES

This application is a continuation-in-part of application Serial No. 08/110,161, filed August 20, 1993, and application Serial No. 07/887,331, filed May 22, 1992, now abandoned. This invention was made with Government support under Grant No. CA 50234 and MH 47680, awarded by the National Institutes of Health. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to methods and compositions useful in inhibiting disorders dependent upon expression of the inducible transcription factor NF-κB.

2. Description of Related Art

The NF-κB transcription activator is a multiprotein complex which can rapidly induce the synthesis of defense and signalling proteins upon exposure of cells to a wide variety of mostly pathogenic agents. Three protein subunits, IκB, p50, and p65, control the biological functions of NF-κB. IκB is a 35-43 kDa subunit which inhibits the DNA-binding of NF-κB and serves to retain NF-κB in an inducible form in the cytoplasm of unstimulated cells. Upon stimulation of cells, IκB dissociates from the inactive complex with p65 and p50. The released p50-p65 complex can then migrate into the nucleus and potently transactivate genes.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1A, B is a Northern analysis of early genes in tax or NF-κB antisense treated cells.

FIGURE 2 is a CAT assay in the presence of NF-κB p65 antisense ODNs.

FIGURE 3 is an electrophoretic mobility shift assay (EMSA) of nuclear extracts obtained from unmanipulated, sense and antisense treated cells.

FIGURE 4 is growth curves of cells treated with tax or NF-κB antisense, *in vitro*. Symbols are mock (No;), sense (SEN; ○) and antisense (ANT; •).

FIGURE 5 shows *in vivo* growth inhibition of B cell line tumors by NF-κB antisense treatment.

FIGURE 6 shows the effect of NF-κB antisense and N-acetyl cysteine (NAC) on mouse survival after LPS challenge.

FIGURE 7 shows serum IL-6 levels after LPS challenge. Error bars represent one standard deviation.

FIGURES 8a and 8b show a Northern blot analysis of NF-κB dependent gene expression in tissues of LPS challenged mice. Lanes 1-9 represent pretreatment for 3 hours with NAC or antisense NF-κB. Lanes 10-18 show the effects of 24 hours pretreatment.

DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention relates to an antisense oligonucleotide sequence which can hybridize to nuclear factor κB (NF-κB) subunit mRNA. This antisense sequence is highly useful for suppressing *in vitro* or *in vivo* NF-κB dependent or associated processes in individuals. Such processes are typically associated with such disorders as those mediated by immune or cytokine responses (for example, septic or non-septic shock) as well as those disorders induced by infectious agents such as retroviruses, more specifically, HIV and HTLV.

The antisense oligonucleotides of the invention are preferably directed to the p65 or p50 subunits NF-κB mRNAs. Most preferably, the antisense oligonucleotides are complementary to the translation initiation nucleic acid sequence of these subunits. In general, the antisense oligonucleotides of the invention are capable of hybridizing to DNA which has the following nucleotide sequence (hu=human; mus=murine):

5'-ATCTTCACCATGGCAGACGAT-3' (hu p50) (SEQ ID NO:1) or
5'-CGGCCATGGACGAACTGTTC-3' (hu p65) (SEQ ID NO:2) or
5'-ATCTTCACCATGGCAGACGA-3' (mus p50) (SEQ ID NO:3) or
5'-TGACCATGGACGATCTGTTT-3' (mus p65) (SEQ ID NO:4).

Most preferred are antisense oligonucleotides having the nucleotide sequence
5'-ATCGTCTGCCATGGTGAAGAT-3' (hu p50 AS) (SEQ ID NO:5) or
5'-GAACAGTTCGTCCATGGCCG-3' (hu p65 AS) (SEQ ID NO:6) or
5'-TCGTCTGCCATGGTGAAGAT-3' (mus p50 AS) (SEQ ID NO:7) or
5'-AAACAGATCGTCCATGGTCA-3' (mus p65 AS) (SEQ ID NO:8).

As a general matter, the oligonucleotide employed will have a sequence that is complementary to the sequence of the target RNA. However, absolute complementarity is not required; in general, any oligonucleotide having

Soc. 106:6077, 1984). The modification to the antisense oligonucleotide is preferably a terminal modification in the 5' or 3' region. Furthermore, recent advances in the production of oligoribonucleotide analogues mean that other agents may also be used for the purposes described here, for example, 2'-methylribonucleotides (Inoue, *et al., Nucleic Acids Res.* 15:6131, 1987) and chimeric oligonucleotides that are composite RNA-DNA analogues (Inoue, *et al., FEBS Lett.*, 215:327, 1987).

Of course, in order for the cell targets to be effectively inhibited by the selected antisense oligonucleotides, the cells must be exposed to the oligonucleotides under conditions that facilitate their uptake by the cells. For *in vitro* therapy this may be accomplished by a number of procedures, including, for example, simple incubation of the cells with the oligonucleotides in a suitable nutrient medium for a period of time suitable to achieve selective inhibition of the cells. For example, where the cell targets of the antisense oligonucleotide of the invention are present in bone marrow cells, procedures can be employed such as those described by Gartner and Kaplan, *Proc. Natl. Acad. Sci. USA*, 77:4756, 1980; Coulombel, *et al., Blood* 67:842, 1986; Meagher, *et al., Blood*, 72:273, 1988; or U.S. Pat. No. 4,721,096 with an optimal concentration of the selected antisense oligonucleotide. These procedures include delivery of the antisense by liposomes or by adenovirus vectors for example. After the marrow cells have been exposed to the oligonucleotide and, in some cases, cultured as described above, they are then infused into the transplant recipient to restore hematopoiesis.

The antisense oligonucleotide of the invention can also be administered to provide *in vivo* therapy to a patient having a disorder which is associated with activation of NF-κB. Such therapy can be accomplished by administering, *in vitro* and *in vivo* as the case may be, a therapeutically effective amount of antisense oligonucleotide, or as further described below, the antisense

administer the antisense oligonucleotide in combination with a precursor of glutathione. The term "in combination" means that the antisense oligonucleotide and the glutathione precursor are administered (1) separately at the same or different frequency using the same or different administration or (2) together in a pharmaceutically acceptable composition. If desired, the antisense oligonucleotide and glutathione precursor can be administered substantially contemporaneously. The term "substantially contemporaneously" means that the antisense oligonucleotide and glutathione precursor are administered reasonably close together with respect to time, for example, simultaneously to within a few hours.

"Pharmaceutical combination" includes intimate mixtures of the two components of the invention, as in classical compositions, and also non-mixed associations, such as those found in kits or pharmaceutical packs.

Antisense oligonucleotide, alone or in combination with glutathione precursor, can be administered in a single dose or can be administered in multiple doses over a period of time, generally by injection. Various administration patterns will be apparent to those skilled in the art. The dosage ranges for the administration of the antisense obigonucleotide of the invention are those large enough to produce the desired effect of suppressing the undesired NF-κB dependent process. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art without undue experimentation. The dosage can be adjusted by the individual physician in the event of any counter indications, immune tolerance, or similar conditions. Those of skill in the art can readily evaluate such factors and, based on this information, determine the particular therapeutically effective concentration of antisense oligonucleotide. or antisense oligonucleotide in

invention in sprays, solution, and the like. Injections and implants are preferred because they permit precise control of the timing and dosage levels used for administration, with injections being most preferred. Antisense oligonucleotide is preferably administered parenterally.

Glutathione precursor according to the present invention can be administered to the animal in any acceptable manner including by injection, using an implant, and the like. Injections and implants are preferred because they permit precise control of the timing and dosage levels used for administration, with injections being most preferred. Glutathione precursor is preferably administered parenterally.

Antisense oligonucleotide and glutathione precursor compositions can be administered in an injectable formulation containing any glutathione precursor and antisense oligonucleotide compatible and biocompatible carrier such as various vehicles, adjuvants, additives, and diluents to achieve a composition usable as a dosage form.

Aqueous vehicles such as water having no nonvolatile pyrogens, sterile water, and bacteriostatic water are also suitable to form injectable antisense oligonucleotides and glutathione precursor formulations. In addition to these forms of water, several other aqueous vehicles can be used. These include isotonic injection compositions that can be sterilized such as phosphate buffered saline, sodium chloride, Ringer's, dextrose, dextrose and sodium chloride, gelatin and lactated Ringer's. Addition of water-miscible solvents, such as methanol, ethanol, or propylene glycol generally increases solubility and stability of the compounds in these vehicles.

Nonaqueous vehicles such as cottonseed oil, squalene, sesame oil, or peanut oil and esters such as isopropyl myristate may also be used as solvent

EXAMPLE 1

ANTISENSE INHIBITION OF TUMOR CELLS *IN VITRO*

A. PREPARATION OF OLIGONUCLEOTIDES

Sense and antisense oligodeoxynucleotides (ODNs) were synthesized by the phosphoramide method on an ABI automated synthesizer (Foster City, California). The phosphothioate (PS) sulfurization modification (Stein, *et al.*, *Nucleic Acids Res.*, 16:3209, 1988) was performed during synthesis, where TETD and acetonitrile were substituted for the usual iodine, pyridine and water during the oxidation step. ODNs were purified according to published procedures (Miller, et al., *J. Biol. Chem.*, 255:9659, 1980).

One set of sequences was selected to be complementary to the transcript encoded by the HTLV-I LTR-tax cassette which had previously been used to generate transgenic mice (Nerenberg, *et al.*, *Science*, 237:1324, 1987). The antisense sequence was GAAGTGGGCCATGTGGA*A*G (SEQ ID NO:9) (location 718-737 on HTLV-I LTR-tax construct) which included the AUG initiation codon (underlined) and sense sequence was CTTCCACATGGCCCAC-T*T*C* (SEQ ID NO:10), the exact complement of the tax-antisense ODN above. Asterisks show sites of PS modification.

An upstream sense primer (AAGCGTGGAGACAGTTCAGG, SEQ ID NO:11, location 423-442) was synthesized as a primer pair for the modified or unmodified antisense primer (location 737-718). Similarly, a downstream antisense oligonucleotide (TTGGCGGGGTAAGGACCTTG, SEQ ID NO:12, location 986-1004) was synthesized to pair with the modified or unmodified sense (location 718-737).

caused metastasis. These tumors attracted high numbers of granulocytes, similar to the original tumors (Nerenberg, *et al.*, *Science*, 237:1324, 1987), presumably caused by high secretion of cytokines. Mice typically die from localized effects of the tumor by 2 months after injection. Other cell lines had similar gene expression phenotypes, but grew more slowly, or required higher numbers of inoculated cells to form tumors. Cells were H-2^B haplotype, and expressed high levels of tax.

C. EFFECT OF OLIGONUCLEOTIDES ON TUMOR CELLS *IN VITRO*

Cells of the B line were cultured in T-75 flasks to 70% confluence. Cells were grown in modified DMEM (Cellgro, Mediatech, Washington, DC) plus 10% heat inactivated fetal calf serum (FCS) (GIBCO, Grand Island, New York) except where indicated. The unmodified or PS modified ODNs were added to a final concentration of 20 μg/cc for 48 hrs. The cells were pre-incubated for 6 hrs in the presence of these ODNs with 10% FCS or without serum prior to transfections. Poly A+ RNA was extracted from tissues and digested with proteinase K and purified by the FastTrack method (Bradley, *et al.*, *Bio-Techniques*, 6:114, 1988) (Invitrogen, San Diego, California). Samples were denatured and run on formaldehyde 1.4% agarose gels followed by blotting onto nylon membrane and UV fixation. Cloned cDNAs (early genes) were used for generation of probes. Probes were labeled via the random primer method with ^{32}P dATP plus dCTP. Specific activities were 1 x 10^9 cpm/mg. The effect of antisense on tax or NF-κB gene expression *in vitro* is shown by Northern analysis in FIGURE 1, A and B, respectively.

Tax specific sense ODNs used as a control, had no effect on cell growth or gene expression (FIGURE 1A lane 2). Previous analyses of tax antisense ODN treated cells demonstrated approximately 10 fold decrease in tax protein production, which led to a corresponding 10 fold decrease in transactivation

The findings above were further confirmed by direct assay of protein binding to NF-kB target sites. Electrophoretic mobility shift analysis (EMSA) of nuclear extracts obtained from unmanipulated, sense and antisense treated cells are shown in FIGURE 3.

The target sequence for EMSA consisted of a double stranded 35-mer:5'-AGCTTCAACAGAGGGGACTTTCCGAGAGGCTCGAG-3' (SEQ ID NO:13) (ODN A). The underlined sequence is identical to the NF-κB consensus in mouse Ig k light chain and to that used by N. Kabrun, *et al.*, *Proc. Natl. Acad. Sci.*, 88:1783, 1991. The second strand was synthesized by hybridization with an 11-mer (ODN B), (complementary to the sequence not underlined) which was labeled at its 3' end by klenow mediated extension in the presence of α-^{32}P dATP. This yielded a double stranded labeled 35-mer. The sequence for the mutant NF-κB was generated from a 35-mer (ODN c) sequence: 5'-AGCTTCA-ACGAGGCGACTTTCCGAGAGGCTCGAG-3'(SEQ ID NO:14). The NF-κB site is underlined with the mutation in small letters. This ODN was hybridized with ODN B and filled using klenow in the presence of unlabeled dNTPs.

For these experiments, 3mg of nuclear extract from untreated (no), sense treated (se), or antisense treated (an) cells (cell line B) were incubated with a double stranded ODN encoding the NF-κB consensus target sequence (Kabrun, *et al.*, *Proc. Natl. Acad. Sci.*, 88: 1783, 1991). 1 x 10^4cpm (approx. 0.86 ng) of labeled ODN was used. Procedures were as published (Kabrun, *et al.*, *Proc. Natl. Acad. Sci.*, 88: 1783, 1991). Three NF-κB specific bands (I, II, III) (Baldwin, *et al. Mol. Cell. Biol.*, 11:4943, 1991) were readily identified. Competition assays are shown in the left panel. The ratios of molar excess of unlabeled consensus ODN (NF-κB) or mutant NF-κB(M) are shown.

Analysis of unmanipulated cells (lanes 1,6) revealed 3 bands of NF-κB complexes similar to those previously described (A. Baldwin, *et al., Mol. Cell.*

Similar results were obtained when the concentration of NF-κB ODN was varied and serum concentration was fixed, or with other tax expressing fibrosarcoma lines. p50 ODNs were slightly less effective at the same concentration but gave a similar profile. Mixtures of both p50 and p65 ODNs had an additive inhibitory effect.

It is likely that additional HTLV-I encoded proteins other than tax are necessary for transformation by the native virus. Thus, tax and NF-κB may play different roles in maintenance of transformation of human lymphocytes than that demonstrated for mouse fibroblasts. To determine the effects of tax and NF-κB antisense ODNs on lymphocytes, the HTLV-I transformed human cell line, MT2 (M. Kozac, *Nature,* 308:241, 1984) was analyzed. FIGURE 2B shows a profile of growth inhibition similar to that seen for the HTLV-I transformed murine cell line MT-2 (Kozac, *Nature*, 308:241, 1984). Cells were grown in 4% FCS in the presence or absence of NF-κB or tax ODNs. Synthesis of new PS ODN was required, as the 5' end of the tax translation initiation target sequence in the transgenic mice varied slightly from that in the native human virus. (The sequence for inhibition of tax in human cells is antisense 5'-TCGTCTGCCATG-GTGAAGAT-3'.) Cells were allowed to grow for up to 20 days. Growth is expressed as absolute cell number in triplicate 6 well dishes. Tax antisense ODNs had no apparent effect on growth of the infected human cell line (left panel), despite significant inhibition of tax protein. In contrast, p65 antisense ODNs profoundly inhibited growth at all time points.

TABLE 1

TIME COURSE ANALYSIS OF NF-κB p65 SENSE AND ANTISENSE TREATED MICE

DAYS AFTER START OF TREATMENT	TREATMENT		
	NONE	SENSE	ANTISENSE
0	348 ± 214[a]	N.D.[b]	N.D.
4	799 ± 343	748 ± 424	260 ± 198
8	1385 ± 774	1264 ± 671	122 ± 84
15	5394 ± 2864	4874 ± 2571	45 ± 28
60	N.D.	7243 ± 3872	<45[c]

[a] tumor weight without capsules (mg)
[b] not determined
[c] undetectable (less than 45 mg)

Clear growth inhibition was seen as early as 8 days after first treatment with profound differences occurring by 15 days. Histologic analysis of these tumors was also performed. Samples obtained from mice treated with sense NF-κB ODN revealed typical morphology characteristic of growing tumors. Treatment with antisense ODN showed focal tumor necrosis with inflammatory infiltrate by 4 days, followed by widespread segmental necrosis by 7 days. By 10 days, fibrotic tissue and tumor capsule with occasional inflammatory cells were all that remained. Beyond 10 days, it was difficult to identify the tumor site. Untreated mice, or those treated with sense ODNs, died between 8 and 12 weeks, while antisense ODN treated mice have been followed for up to 5 months without evidence of recurrence of tumors. In none of these mice were ODN injections given beyond the first 9 days.

The present studies reveal striking similarities between the effects of tax on mouse fibroblasts and HTLV-I or II virus on human T-cells. Though tax is necessary for transformation of mouse fibroblasts or human T-cells, the present

EXAMPLE 3

ANTISENSE NF-κB ODNs PREVENT LPS MEDIATED DEATH

In order to compare the effects of NAC or NF-κB AS-ODNs on the toxic manifestations of LPS induced septic shock, survival rates for the different treatment groups of mice were compared. This data is summarized in FIGURE 6.

LPS-induced lethality. In vivo LPS challenge was performed according to the modified methods of Broner *et al*, *Critical Care Medicine* 16:848, 1988; and Peristeris, *et al.*, *Cellular Immunology*, 140:390, 1992. A total of 35 C57Bl/6 mice (body weight 25-35g) were studied in three treatment groups: 1) 10 mice were treated with LPS followed by two mock injections of saline. 2) 10 mice were pretreated with NAC 12 hours and 1/2 hour prior to innoculation with LPS. 3) 10 mice were pretreated with NF-κB antisense ODNs 20 hours and 1/2 hour prior to LPS, and 5 mice were treated 20 and 1/2 hours prior to LPS with an irrelevant HTLV-I specific antisense ODN (Kitajima, I., *et al., J. Biol. Chem.* 267: 25881, 1992) as a control.

LPS (*Escherichia coli* 055:B5. Sigma, St. Louis., MO) was given as a single dose by intraperitoneal (ip) injection. Optimal results were obtained when a total dose of 100-150 μg (approx 5μg/g body wt.) was administered in 0.3 ml of sterile, pyrogen-free saline. Qualtitatively similar results were obtained when sepsis was induced with 75 or 200 mg, with delayed or accelerated sickness, respectively. Two experiments were performed with 5 mice per group. NAC (Sigma, St. Louis. MO) was given intraperitonealy at a dose of 0.27 mg/g of body weight at a single site. Antisense NF-κB p65 ODN (sequence 5'-AAACA-GATCGTCCATGGTCA-3') was 3' terminal phosphorothioate (PS)-modified (Kitajima, I., *et al., Science*, 259:1523, 1993; and Winer, B.J. 1971. Statistical Principles *In* Experimental Design. McGraw-Hill New York, NY), as this has

was 70%. Treatment with tax antisense or NF-κB sense control ODNs (Kitajima, I., *et al., Science.* 258:1792, 1992; Kitajima, I., *et al., J. Biol. Chem.* 267:25881, 1992) gave results identical to the untreated control.

EXAMPLE 4

SERUM LEVELS OF IL-6 ARE DECREASED BY NF-κB ANTISENSE ODNs WHEN ADMINISTERED PRIOR TO SEPTIC SHOCK

The results of Example 3 suggest NF-κB antisense inhibition dramatically reduces LPS induced lethality. In order to confirm that this is a specific consequence of NF-κB inhibition, the level of serum IL-6 was measured. Previous studies have demonstrated that IL-6 serves as an accurate indicator for NF-κB activation as this is a primary transcriptional activator of this cytokine (Liberman, T., *et al., Cell. Biol.* 10:2327, 1990). In addition, IL-6 is an important mediator of catastrophic immune responses such as inflammatory nephropathy (Horii, Y., *et al., J. Immunol.* 143:3949, 1989; Rugo, H.S., *et al., J. Clin. Invest.* 89:1032, 1992), may modulate immunoreactivity of tumors (Tabibzadeh, S.S., *et al., Am. J. Pathol.* 135:1025, 1989), and has been strongly implicated in the pathogenesis of septic shock (Ulich, T.R., *et al., J. Immunol* 146:2316, 1991; Troutt, A.B. *et al., J. Cellular Physiology*, 138:38, 1989).

Assay for IL-6 protein in serum. Sera from mice within each treatment group were obtained at 0, 1, 4, 8, and 20 hrs after LPS administration. Serum IL-6 was determined by an enzyme-linked immunosorbent assay (ELISA) according to the method of Pruslin et al. (Pruslin, F.H., *et al., J. Immunol. Method* 137:27, 1991) using rat anti-mouse monoclonal antibodies to IL-6 (Pharmingen, San Diego, CA). The detection limit for this assay was 100 pg/ml. Statistical differences between treatment groups were analyzed by the ANOVA test for

dependent on activation state (Kitajima, I., *et al., J. Biol. Chem.* 267:25881, 1992). Therefore, the effects of NF-κB antisense on IL-6 in individual tissues in the presence or absense of LPS activation were evaluated. IL-6 mRNA expression was monitored because previous studies have shown that its expression may be pleomorphically induced in a large number of tissues (Ulich, T.R., *et al., J. Immunol* 146:2316, 1991; Troutt, A.B. *et al., J. Cellular Physiology*, 138:38, 1989) and can therefore be used to assess NF-κB inhibition in variety of tissues.

Northern blot analysis. Kidney, liver, lung, spleen and salivary glands from representative mice of each group were rapidly frozen in liquid nitrogen and crushed with a sterile pestle while frozen. mRNA was extracted using the Fast Track method (Bradley, J.E., *et al., BioTechniques* 6:114, 1988) (Invitrogen, San Diego, CA). The positive control for murine gene expression (TNF-α, IL-6, MHC class I and GM-CSF), was mRNA obtained from an HTLV-I tax expressing mouse fibrosarcoma cell line (Kitajima, I., *et al., J. Biol. Chem.* 267:25881, 1992). 7 mg mRNA per lane was loaded and electrophoresis was performed on formaldehyde 1.2% agarose gels. Samples were blotted onto nylon membrane and fixed by UV crosslinking. Murine cDNA probes of MHC class I & II and GM-CSF were labeled via the random primer method with [^{32}P]dATP. IL-6 TNF-α and actin probes cloned in pUC, pBluescript or pGem plasmids were labeled with [^{32}P]dATP by polymerase chain reaction using universal primers complementary to regions flanking the linkers. Specific activities of probes were in excess of 1X10^{9} cpm/mg.

mRNAs were extracted from each tissue, 3 hrs after LPS injection. In Figure 8, the lanes were as follows: **B** is an HTLV-I tax transformed cell line which expresses high levels of NF-κB and serves as a positive control. **No** is obtained from mock (saline treated) mice. **LPS**, obtained after no pretreatment. **NAC**, is from pretreated mice (8 mg x 2). **NF** is from an animal

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE SCRIPPS RESEARCH INSTITUTE

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(v) COMPUTER READABLE FORM:
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(2) INFORMATION FOR SEQ ID NO:1:

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: p50 S mus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATCTTCACCA TGGCAGACGA 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: p65 S mus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAACAGTTCG TCCATGGCCG 20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: p50 AS mus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCGTCTGCCA TGGTGAAGAT 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: p65 AS mus

(ix) FEATURE:
(A) NAME/KEY: CDS

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTTCCACATG GCCCACTTC 19

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AAGCGTGGAG ACAGTTCAGG 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTGGCGGGGT AAGGACCTTG 20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGCTTCAACG AGGCGACTTT CCGAGAGGCT CGAG 34

7. The antisense oligonucleotide of claim 1, wherein the antisense nucleic acid is DNA.

8. The antisense oligonucleotide of claim 1, wherein the antisense nucleic acid is RNA.

9. The antisense oligonucleotide of claim 1, which is selected from the group consisting of:
5'-ATCGTCTGCCATGGTGAAGAT-3' (hu p50 AS) (SEQ ID NO:5),
5'-GAACAGTTCGTCCATGGCCG-3' (hu p65 AS) (SEQ ID NO:6),
5'-TCGTCTGCCATGGTGAAGAT-3' (mus p50 AS) (SEQ ID NO:7), and
5'-AAACAGATCGTCCATGGTCA-3' (mus p65 AS) (SEQ ID NO:8).

10. A recombinant DNA sequence which upon, transcription encodes an antisense oligoribonucleotide that hybridizes to an NF-κB subunit mRNA and that hybridizes to a DNA sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein the antisense inhibits translation of the mRNA.

11. A method of suppressing NF-κB dependent processes in an individual which comprises administering a therapeutically effective amount of the antisense oligonucleotide of claim 1.

12. The method of claim 11, wherein the antisense oligonucleotide is from about 8 to about 40 nucleic acid residues in length.

13. The method of claim 11, wherein the antisense oligonucleotide is chemically modified.

21. The method of claim 20, wherein the antisense oligonucleotide and glutathione precursor are administered substantially contemporaneously.

22. The method of claim 20, wherein the glutathione precursor is an acylcysteine.

23. The method of claim 22, wherein the acylcysteine is N-acetylcysteine.

24. A pharmaceutical combination comprising the antisense oligonucleotide of claim 1, in combination with a glutathione precursor.

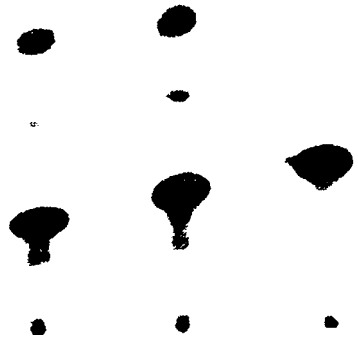
25. The method of claim 11, wherein the NF-κB dependent process is septic shock.

26. The method of claim 11, wherein the NF-κB dependent process is tissue specific.

27. The method of claim 26, wherein the tissue is selected from the group consisting of kidney, liver, and spleen.

28. A method of monitoring the effectiveness of suppressing NF-κB dependent processes in the tissue of an individual after administering a therapeutically effective amount of the antisense oligonucleotide of claim 1, comprising detecting the level of cytokine production in a tissue before and after the antisense therapy.

29. The method of claim 28, wherein the cytokine is IL-6.



	No oligo.	Sense	Antisense
LTR-CAT % conversion	6.5	6.4	0.3

FIG. 2

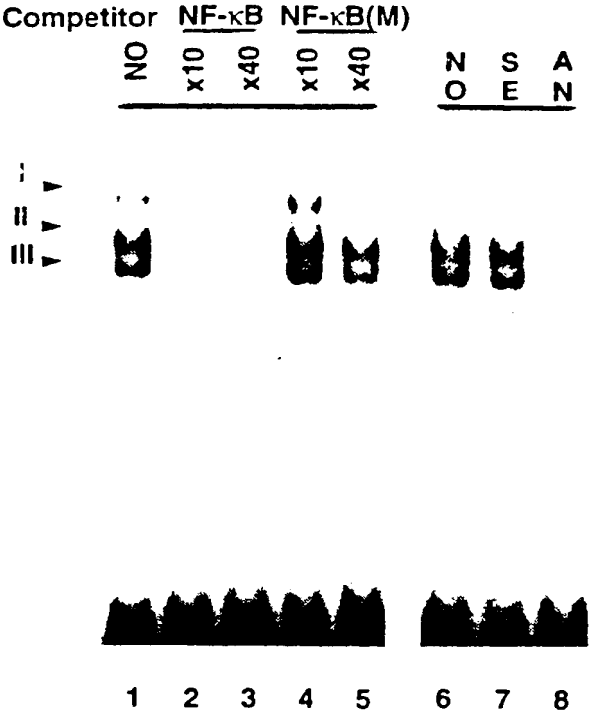


FIG. 3

Sense

FIG. 5A

Antisense

FIG. 5B

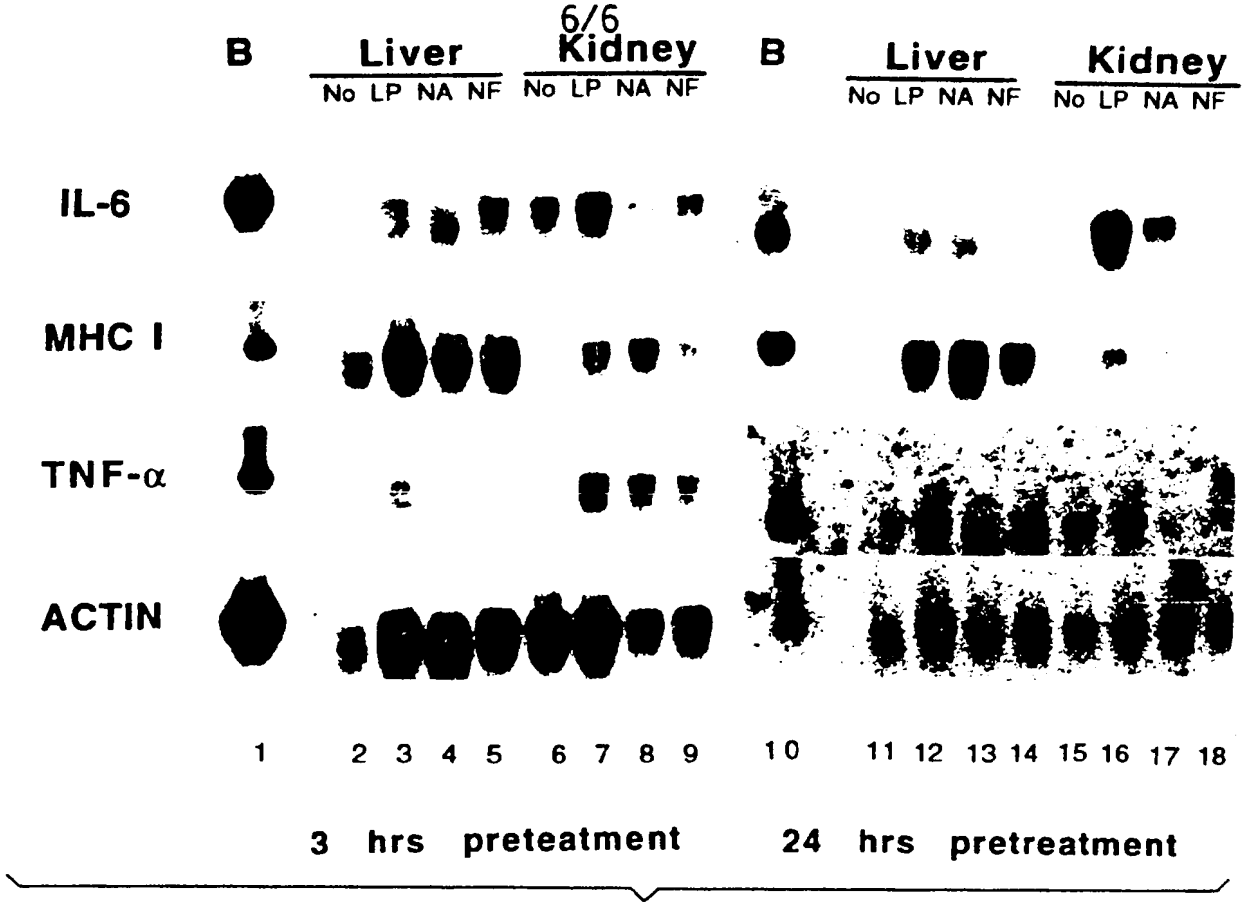

B
Liver
No LP NA NF
Kidney
No LP NA NF
B
Liver
No LP NA NF
Kidney
No LP NA NF
IL-6
MHC I
TNF-α
ACTIN
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
3 hrs preteatment
24 hrs pretreatment

FIG.8a

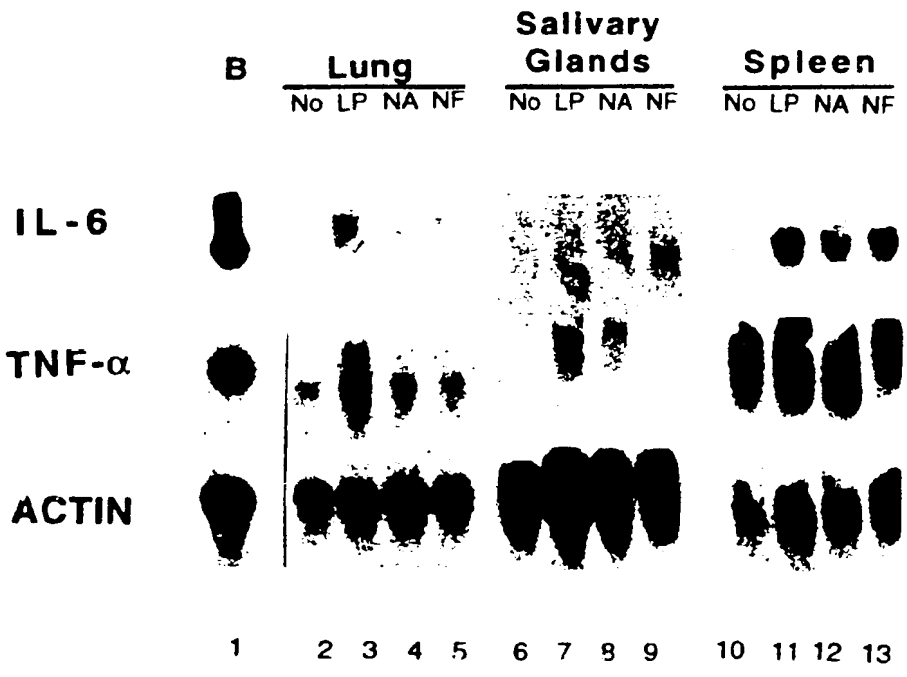

B
Lung
No LP NA NF
Salivary Glands
No LP NA NF
Spleen
No LP NA NF
IL-6
TNF-α
ACTIN
1 2 3 4 5 6 7 8 9 10 11 12 13

FIG.8b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/09350

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Chemical Reviews, Volume 90, No. 4, issued June 1990, E. Uhlmann et al, "Antisense Oligonucleotides: A New Therapeutic Principle", pages 543-584, see the entire document.	1-29
Y	Proceedings of the National Academy of Science, USA, Volume 87, issued December 1990, F.J.T. Staal et al, "Intracellular thiols regulate activation of nuclear factor κB and transcription of human immunodeficiency virus", pages 9943-9947, see the entire document.	20-24
Y	Biochimica et Biophysica Acta, Volume 1072, issued 1991, P.A. Baeuerle, "The inducible transcription activator NF-κB: regulation by distinct protein subunits", pages 63-80, see the entire document.	1-29